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Development of an assay using real time cell analysis for the measurement of equid herpesvirus 1 specific neutralizing antibody in horses after experimental infection or field vaccination

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Study design: Randomised double-blinded vaccination/challenge study.

Methods: Clinical and immunological outcomes of primary experimental infections of horses with Ab4, or Ab4ΔORF2 compared to non-infected controls were assessed (n=8/group). All horses were challenged with EHV-1 Ab4 intranasally nine-months later. Clinical outcomes and correlates of protection were analysed.

Results: Primary infection with the vaccine candidate Ab4ΔORF2 reduced fever and minimised nasal virus shedding after infection compared to Ab4, while Ab4ΔORF2 established cell-associated viraemia similar to Ab4. The horses' immune responses were similar after infection with Ab4 or Ab4ΔORF2 and were dominated by EHV-1-specific antibodies in nasal secretions and serum. After the challenge infection, previously uninfected horses (Control/Ab4) displayed fever and clinical disease, shed high amounts of virus, and developed viraemia. In contrast, 5/8 (Ab4ΔORF2/Ab4) and 3/8 (Ab4/Ab4) horses previously infected with Ab4ΔORF2 or Ab4, respectively, were fully protected from Ab4 challenge infection as indicated by the absence of fever, clinical disease, nasal virus shedding, and viraemia. All of these outcomes were significantly reduced in the remaining, partially protected 3/8 (Ab4ΔORF2/Ab4) and 5/8 (Ab4/Ab4) horses. Protection was linked to pre-existing and rapidly boosted nasal EHV-1-specific antibody responses, that likely neutralised EHV-1 Ab4 at the respiratory entry site. Protection was also associated with EHV-1-specific serum antibodies pre-challenge.

Main limitations: A limited number of horses and no cell mediated immunity were studied. However, the protective nature of pre-challenge intranasal antibodies agreed with a previous vaccination/challenge study [1].

Conclusions: Robust protection from challenge infection emphasises Ab4ΔORF2 as a vaccine candidate. EHV-1-specific antibodies are strong correlates of protection against EHV-1 infection.

Reference

[1] Perkins, G., Babasyan, S., Stout, A.E., Freer, H., Rollins, A., Wimer, C.L. and Wagner, B. (2019) Intranasal IgG4/7 antibody responses protect horses against equid herpesvirus-1 (EHV-1) infection including nasal virus shedding and cell associated viremia. *J. Virol.* **531**, 219–232. <https://doi.org/10.1016/j.virol.2019.03.014>

Ethical animal research: The animal protocol was approved by the Institutional Animal Care and Use Committee at Cornell University (protocol #2011-0011).

Informed consent: Not applicable.

Competing interests: None declared.

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79 | Development of an assay using real time cell analysis for the measurement of equid herpesvirus 1 specific neutralizing antibody in horses after experimental infection or field vaccination

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Background: Equid herpesvirus-1 (EHV-1) is one of the most important horse pathogens, which induces respiratory disease, myeloencephalopathy, abortion or still-birth. The measurement of EHV-1 neutralising (EHV-1-VN) antibodies using a neutralisation assay (NA) gives an indication of the immune response of horses in the field.

Objectives: To develop a real time neutralisation assay (RTNA) using the xCELLigence[®] technology for the measurement of EHV-1-VN antibodies in serums from EHV-1 experimentally infected or vaccinated horses.

Study design: Assay development and field trial.

Methods: The RTNA based on E. Derm cells was developed using serums (n=48) taken daily (from Days 0 to 18 post-infection) from ponies experimentally infected with a field EHV-1 strain. This method was further evaluated using field serums (n=63) taken during an EHV-1/-4 vaccination campaign. RTNA results were compared to those obtained with the conventional EHV-1 NA on RK13 cells. Two-fold serial dilutions of serums were incubated with a defined concentration of the KyD EHV-1 strain before infection of E. Derm cells. The normalised cell index was monitored pre- and post-infection using the xCELLigence[®] technology. Conventional NA was performed using the same experimental methodology on RK13 cells and cytopathic effects were measured 72 hours post-infection.

Results: Serum titres which induced 50% of EHV-1 neutralisation (NT₅₀) were determined using a dose-response approach. The increase of the EHV-1 NT₅₀ was similar to the increase in titre measured with the conventional NA (R²=0.79). Seroconversion was detected between Days 0 and 18 post-infection for all infected ponies with both methods. The NT₅₀ values measured for EHV-1/-4 vaccinated horse serums were also positively correlated with conventional NA titres (R²=0.83).

Main limitations: EHV-1/-4 cross-reactivity will require further investigation. Each assay used different cells.

Conclusion: While the conventional NA is time consuming, requires subjective measurements and provides semi-quantitative discrete

ranked titres (e.g. 4, 8 etc.), the EHV-1-VN RTNA is an automated, sensitive and objective measurement that reports continuous titres.

Ethical animal research: All experimental procedures were approved by the Loire Valley ethical review board (CEEA VdL, committee number 19).

Informed consent: Informed consent was given by the horses' owners.

Competing interests: None declared.

Sources of funding: Fonds Eperon OVERLORD N12-2017, Normandy County Council (17E01598/17EP04324), the IFCE (Institut Français du Cheval et de l'Équitation) grant number 2017-008, CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational programme 2014-2020.

80 | Detection of *Equid herpesvirus-1* in serum samples collected from infected horses

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Background: Abortion and myeloencephalopathy are caused by *Equid herpesvirus-1* (EHV-1) infection as a consequence of its transmission to susceptible organs by viraemia. Since EHV-1 circulates in the bloodstream in a cell-associated manner, serum samples collected from infected or febrile horses are rarely used for virus detection.

Objectives: To determine the usefulness of horse serum samples for the detection of EHV-1.

Study design: Assay assessment.

Methods: Archived sera and peripheral blood mononuclear cells (PBMCs) collected daily from three horses that had been experimentally inoculated with EHV-1 during the subsequent two-week observation period [1] were investigated. Acute-phase serum samples collected from 40 febrile ($\geq 38.5^\circ\text{C}$) horses, including 11 serologically confirmed field EHV-1 cases were also examined. Real-time PCR was used to detect EHV-1 in the samples.

Results: EHV-1 was detected in experimental PBMC and serum samples for 6 to 7 days (from post-infection day [PID] 5 or 6 to 11 and sporadically in one sample at PID 1) and 5 to 7 days (from PID 5 or 7 to 11), respectively. Six of 11 acute-phase serum samples collected from field EHV-1 cases were positive for the virus, whereas the rest of the field sera were negative.

Main limitation: The presence of cell-free intact particles of EHV-1 in horse sera was unclear.

Conclusions: EHV-1 was detected almost simultaneously in PBMC and serum samples collected from experimentally infected horses. Additionally, more than half of the field acute-phase sera collected from EHV-1 infected horses tested positive for the virus, which suggests that the pyrexia observed in these horses was caused by

viraemia. These results show that serum samples collected from EHV-1 infected or febrile horses can be used to detect the virus if PBMC samples are not available.

Reference

[1] Bannai, H., Nemoto, M., Tsujimura, K., Yamanaka, T., Kokado, H., Kondo, T. and Matsumura, T. (2018) Comparison of protective efficacies between intranasal and intramuscular vaccination of horses with a modified live equine herpesvirus type-1 vaccine. *Vet. Microbiol.* **222**, 18–24.

Ethical animal research: This study was approved by the Research Planning and Ethics Committee of the Equine Research Institute with accession number 2018-3263-07.

Informed consent: Owner consent was obtained for all field samples.

Competing interests: None declared.

Source of funding: Japan Racing Association.

81 | Generation of EHV-1 pseudotype virus for cell tropism studies and virus-neutralising assays

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Background: *Equid herpesvirus-1* (EHV-1) can cause respiratory disease, abortion, neonatal death and myeloencephalopathy. Thus, EHV-1 represents a threat to the equine industry. EHV-1 exhibits 12 glycoproteins on its surface envelope, but those important for cell entry/host immune responses remains partially unclear. To investigate the contribution of these glycoproteins, pseudotype viruses (PVs) may provide a useful study tool.

Objectives: Generate high titre EHV-1 PV particles for cell tropism studies and develop tests for virus-neutralising (VN) antibody detection in naturally/experimentally infected horses.

Study design: Assay development.

Methods: 5 EHV-1 glycoprotein gene sequences were obtained from an aborted fetus strain isolated during a large EHV-1 outbreak in France in 2010. Sequences were synthesised and subcloned into expression vectors and employed in lentivirus PV generation. PVs were utilised in a Pseudotype Virus Neutralisation Test (PVNT), a sensitive technique to measure levels of specific VN antibodies. Serum samples (n=48) tested were taken longitudinally (Days 0 to 18 pi) from ponies experimentally infected with EHV-1, compared with uninfected controls (n=4). Plasmids expressing PV components' genes were co-transfected into HEK293T/17 using polyethylenimine (PEI). PV production and quantification were assessed by fluorescence and luminescence, respectively. For PVNT, two-fold serial dilution of equine sera were incubated with PV and target cells. As for